PHYSIOLOGICAL STUDIES OF OAT SMUTS

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INTRODUCTION

Loose and covered smuts of oats are caused by Ustilago avenae (Pers.) Rostr. and U. kolleri Wille, respectively. These two species have been identified from all oat growing areas of the world. The loose smut spores adhere in a loose mass to the central axis of the inflorescence and are readily dispersed by wind. Covered smut spores are produced in balls and are closely covered by glumes, hence are not easily disseminated by wind. These morphological characteristics may account for the higher distribution, in nature, of loose smut than covered smut. As a result, more extensive investigations have been made on the former. Loose smut has been reported from sixty-one countries in some of which the disease constitutes a hazard to economic oat production (19). In the United States estimates indicate that in some years loose smut destroys 40 to 50 million bushels of oats. The heaviest damage occurs in the South Atlantic states. Covered smut is not as prevalent as loose smut in this region (53).

Oat smuts are controlled to a great extent by development of resistant varieties of oats, therefore control is limited by the evolution of new races. Since the origin of new races is common in nature, many resistant varieties exhibit susceptibility soon after they are released for commercial production. New races have been described frequently in U. avenae (49). The host-parasite interactions, as manifested in nature in the form of pathogenicity, demonstrate the phenomenon of pathogenic specialization. Differential virulence of certain smut races on a single host differential

variety indicates that there are specific genetic factors which govern pathogenicity. Similarly, the differential reaction of two host varieties to a single pathogenic race connotes the presence of different genetic factors in the host for susceptibility and resistance. Therefore, the adverse effects of oat smut races are exhibited in nature on particular host varieties as a result of specific contributory genetic factors of pathogen as well as host. Investigations pertaining to host reaction and pathogenic specialization could solve many of the problems of smut control.

In recent years the tendency to grow oat varieties with widely different genetic make up in southern United States may explain the reports of a number of loose smut races from this region; therefore the smut problem in this area has become complex. It was found that southern smut collections did not react normally at Pullman, Washington, nor were the standard host testers used at the Regional Smut Research Laboratory at Pullman adaptable for use at Gainesville, Florida. Therefore, it was necessary to initiate an independent study on the variability and distribution of southern oat smuts (61). Testing 5 smut collections from the South Atlantic States, workers successfully selected adapted varieties that differentiated 5 cultures. These studies were extended in an attempt to compare the pathogenic pattern of these collections with that of previously described races. The 5 collections from the South Atlantic States were subsequently designated as races, $A1^4$, $A1^{4B}$, A20, A21 and A22 (62).

The present studies were carried out with races $A1^4$, $A1^{4B}$, A20 and A22 of U. avenae and collections M5 and M11 of U. kolleri. The primary

objective of this study was to determine pathologic, physiologic and genetic differences between loose and covered smut species that exhibit wide and narrow host ranges. Attempts to grow them on different nutrient media at varying temperatures were made to establish optimum growth conditions. Studies were conducted to determine the effect of culture media and spore age on the degree of infection incited by interracial hybrids. The effect of loose and covered smut infection on the growth rate of selected oat varieties was also determined.

Inheritance of host reaction to 2 loose smut races was determined under field conditions.

Investigations were carried out from the viewpoint of determining whether or not inter or intraspecific biochemical differences exist among the loose and covered oat smuts. These studies were designed to demonstrate chemotaxonomic relationships among the oat smuts. These studies involved the analysis of free and bound amino acids as well as reducing sugars isolated from the chlamydospores of 5 smut cultures. The effect of host variety on the amino acid content of chlamydospores was also determined.

REVIEW OF LITERATURE

The fundamental characteristics of smut fungi were noted very early in the history of plant pathology as evidenced by many classical studies. The studies from the very beginning were concentrated on cereal smuts since these pathogens have been more or less a problem in growing these food crops. By the middle of the sixteenth century, some people categorized smut diseases differently from rusts, although no apparent distinction was made among various smuts. They were all considered in early Greek and Roman literature to be a diseased condition of the host, but of a non-parasitic origin. Camerarius (1709), distinguished wheat bunt from loose smuts of wheat, oats and barley (23). Tillet (1755) in his contribution on bunt of wheat, Tilletia caries, disproved the earlier traditional thinking in regard to non-parasitic nature of smuts (102). He recognized the contagious systemic nature of smut pathogens. Schraeber (1758) denied Tillet's conclusion regarding the infectious nature of smut (23). Benvenuti (1763), like several others of that century, indicated that smut was an advanced stage of rust (23). Gleichen (1781), while working in Germany, demonstrated the seed-transmissible nature of bunt of wheat. This was the first record on the basis of his experimental evidence obtained from seed inoculation tests. Prevost (1807) established the fungal nature of smuts, from his investigations on wheat bunt. He observed the spore germination in Tilletia caries (D.C.) Ful. and Ustilago carbo. He also proved the parasitic nature of bunt (67). In addition to these biological

characteristics Prevost demonstrated the effectiveness of copper compounds for wheat bunt control. As late as 1801, Persoon for the first time officially recognized smuts as fungal organisms. De Candolle (1815), recognized the fungal nature of wheat bunt and named the organism Uredo caries (17). In addition to Prevost's contributions, the Tulasne brothers (1847, 1854) also demonstrated spore germination of the bunt fungus; moreover, they also tried to interpret primary and secondary sporidia (103, 104). Berkeley (1847) for the first time explained the pathologic nature of smut and described the fusion of primary sporidia in pairs (7). De Bary (1853) was also able to demonstrate the sexual significance of sporidial fusion in smuts. This process was named "conjugation" by him. His interpretations were based on the experiments conducted with several genera of smut fungi (16). Extensive investigations by Brefeld for the period ranging from 1877 to 1912 contributed much to the development of present fundamental knowledge of smuts (23). He criticized the term "conjugation" for fusing sporidia as earlier used by De Bary. Brefeld pointed out that sporidia are also capable of growing vegetatively on the proper nutrient media in addition to their capacity of sexual genetic fusion; thus they are analogous to conidia.

Although Hallier (1867) was the first to germinate smut spores by use of nutrients, the real pioneer in the most important study of development of smut fungi on artificial media was Brefeld (1883) (11). Dangeard (1892, 1893, 1894) made a series of cytological studies in regard to the sexual phase of smuts, particularly Ustilago entyloma and Urocystis species. He explained the gametic nature of haploid nuclei which fuse to form a diploid spore (13, 14, 15).

After the morphology and life history of these organisms were well

described, a trend towards the determination of physiologic and genetic characteristics developed. Kniep (1919) made systematic studies on Ustilago violacea (Pers.) Roussel and discovered the heterothallic nature of smuts. He demonstrated that sporidial fusion occurred only between certain sporidia, which were sexually different (23, 57). Goldschmidt in 1928 demonstrated the artificial production of hybrids between physiologic races. He concluded that differences in pathogenicity in U. violacea were controlled by single Mendelian factors (28). In loose and covered smuts of oats, certain basic characters are governed by genetic factors which attain significant relationships for species dynamics (50).

Hanna and Popp (1930) and Holton (1933), on the basis of their investigations with the intrageneric cross, U. avenae X U. kolleri, reported loose smutted panicle to be dominant over the covered type (35, 43). Holton (1932) reported a buff mutant of U. kolleri and found the hyaline-smooth spore characters to be inherited recessively in crosses with parent species and U. avenae. He also found that, among all the crosses with U. avenae, the echinulate spore wall character of this species was dominant over the smooth spore wall of U. kolleri (42).

Oat smuts, like other smut species, are facultative parasites; i.e., they can be grown on artificial culture media, provided the chlamydospores are viable. It has been recognized that environment, as well as genetic characters, affect the spore longevity in smut fungi (23). Sampson (1928) found that the state of maturity affects the longevity of oat smut. It is well known that in all smut fungi mature spores maintain their viability for a longer time than less mature spores of the same species. Fischer (1936) established the limit of longevity of U. avenae as 13 years (21).

Hallier (1867) first used nutrients in spore germination studies but the real contributions toward the development of the smut fungi on artificial media were made by Brefeld (1883) (11, 34). He described and illustrated the growth and sporidial budding of many smut species, for the first time. He also demonstrated that smuts can be continuously and indefinitely propagated on culture media. The culture characters of oat smuts, as with other culturable organisms, is a function of the genetics of the organisms and composition of the substratum. Additional culture studies of other Ustilago species were conducted by Herzberg (1895) Appel and Riehm (1911), on U. nuda and U. tritici (3, 41). Maire (1898) and Gruss (1902) on U. maydis (30, 63). Since then, considerable importance has been given to the cultural aspects of the study of smuts by use of liquid as well as solid culture media composed of a variety of nutrient sources. The medium that promotes luxuriant and rapid growth, with maximum production of sporidia for inoculation purposes is most desired.

Extensive culture studies with 20 different media and about 20 different smuts have revealed the general superiority of potato dextrose agar and Blakeslee's agar. In general, most luxuriant growth results from the richest media. Potato dextrose agar proved unsatisfactory for some collections (23). The presence of some sugars makes considerable difference in the growth response. However, certain sugars, e.g. dextrose, sucrose, and maltose alone are not conducive for good growth. From the standpoint of rapidity of growth, luxuriance and production of sporidia, Blakeslee's agar has been found satisfactory. In a study pertaining to the nutritional requirements of certain Ustilago species, Sartoris (1924) found that maltose was definitely beneficial in

stimulating rapid budding of sporidia. Knlep (1921) was among the first workers to use malt extract in culture of smut fungi. Its superiority was also indicated by Sartoris (1924) (58, 96). Malt extract has been reported to have the further advantage of being a source of desirable minerals (23). Ranker (1930), Sartoris (1924), Zscheile (1951) and Haskins (1950) developed special synthetic media for growth of specific fungi.

In regard to solid and liquid media, the latter is essential for carrying out quantitative growth studies; however, culture characteristics of smut fungi can be best studied on solid media. Except for some specific physiological studies, solid media are equally as good as liquid media. The only disadvantage with agar, as pointed out by Zscheile (1951), is that it carries substances of unknown nutritive value which might make it undesirable for critical studies (113). Haskins (40) while working with Ustilago zeae, grew his cultures in liquid media which were shaken constantly at 30° C. He reported a remarkable increase in growth using liquid shake cultures.

Temperature, like many other environmental factors, affects the rate of germination and growth of smut fungi. The temperature range for spore germination of Tilletia caries and T. contraversa was found to be 2° - 28° C, the optimum being 15° - 20° C (Slang 1956). Rate of spore germination was reported to be slower at low temperatures (96). Dietz (1956) studied the effects of medium and temperature upon chlamydospore germination of Ustilago spegazzinii var. agrestis. From his findings, which involved the use of 3 smut collections of U. spegazzinii and 6 different media, the cardinal temperatures for spore germinations were determined to be: minimum 10° C, optimum 30° C,

and maximum less than 35° C. It was concluded that a minimum of 18 hours was required before spores began to germinate (18). However, in the majority of smut species, spore germination can be obtained at ordinary room temperature, approximately 18° - 20° C (23). Herzberg (41) found the cardinal points of temperature for spore germination of U. avenae and 4 other smuts to be: minimum between 5° and 11° C, optimum 22° to 30° C, and maximum 30° to 35° C. Jones (1923), in his studies related to the determination of effect of temperature upon spore germination of U. avenae, found the minimum temperature to be 4 - 5° C, the optimum 15° - 25° C, and the maximum between 31° and 34° C. The spores were germinated in beef broth at a series of temperatures ranging from 4° to 35° C. Jones also observed that under similar environmental conditions, the minimum and optimum for sporidia production were the same as for germination, but the maximum was somewhat lower, ranging from 29° to 30° C (56).

Along with this extensive knowledge of growing smuts in culture, attempts were made to determine whether these organisms can be made to complete their life cycle on culture media (94). Brefeld (23) made such studies on many smuts. Gruss (1902) made observations on smut spores produced in culture media by Ustilago maydis (30), and a similar report was made by Potter (1914) for Sphacelotheca reiliana (66). In U. avenae, Fleroff (1923) made investigations on the production of chlamydospores on artificial media (24). Wang (1938), Sartoris (1924), Rump (1926), Schaffnitt (1926), Rodenhiser (1926), (1928), Koudelka (1934), Leach and Ryan (1936), and Lowther (1946) also made contributions to the development of cultural techniques for a number of Ustilago species, parasites of grasses and cereals (23, 59, 60, 86, 87, 88, 94, 109).

A number of workers have made expanded studies on various smut species and were able to distinguish physiologic races under cultural conditions (23, 87). Differences in cultural characters are expressed to be phenomena of specialization, to some extent, in U. avenae. But, in oat smuts, physiologic specialization studies on culture media have not received much attention in recent years. Unless pathogenic or host reaction is included, such investigations are usually disregarded. However, Rodenhiser (87) from his studies concluded that cultural characters of U. avenae, derived from multichlamydospores, were adequate for differentiating races. These studies also included U. kolleri. Rodenhiser emphasized that the differentiating cultural characters could only be distinguished by growing smuts on appropriate nutrient media. Utter (1938) made an extensive study with a race of U. avenae in regard to the cultural variants and their pathogenic effect on certain oat differential varieties. Utter's results appear to be more dependable, since he did not consider cultural characters only, to provide an authoritative source of definite identification of smut races (105).

The most predominant symptom of oat smuts on the host plants is the presence of black sori. There are some other effects of oat smut infection on hosts that are not well known. Such modifications are believed to have some relationship to physiological changes, but they have not been studied specifically. Talieff and Grigorovitch (1923) observed noticeable reduction in the height and diameter of the culms of a highly susceptible oat variety as an effect of severe infections of U. avenae (23). Welsh (1932) observed that U. avenae and U. kolleri have a tendency to dwarf the host and that this effect is more pronounced in the latter species (110).

Adverse effects of oat smut infection are not only found in susceptible varieties; they influence the growth patterns of resistant varieties as well. Stevens (1936) studied the so-called "latent infection" (disease without sporulation) in a highly resistant variety, Markton, to both covered and loose oat smuts. He determined that the resistant variety suffered reduced stands and revealed poor yields as a result of smut inoculation (97). Hubbard and Stanton (1934) performed experiments to determine the influence of covered oat smut infection on plant vigor and other characters. Three smut resistant varieties and 1 susceptible oat variety were used. It was found that yield, as well as height of plants, was reduced in both susceptible and resistant varieties; and that infection retarded the date of heading of both resistant and susceptible varieties (54). Zade (1932) described an over-all weakening effect in smut infected plants (112).

In the smut diseases, host reaction to certain pathogenic races is genetically controlled. Many observations (22, 23, 50) on varietal resistance have been made. Studies on the inheritance of host reaction are necessary to develop smut-resistant varieties. The earliest studies designed to test the differential reaction by artificial inoculations of U. avenae and U. kolleri among oat varieties were those initiated by Tubeuf in 1901. Rose (1903) was one of the first to indicate that oat varieties differ in their reaction to smut. He found that 63 varieties gave significant differential reactions, as expressed by smut percentages ranging from 0 - 17 (23). Extensive testing for differential host reaction against oat smut fungi was continued by Reed in 1920 (69). Reed studied 154 oat varieties from 7 oat species. Varying degrees of

reactions were exhibited by varieties of all species, with the greatest range in Avena sativa. These results were confirmed by Reed et al. (1925) and Gaines (1925) in the United States. Rosenstill (1929) and Voss (1939) in Germany; Crepin et al. (1938) in France; Bose and Mundkur (1941) in India; Aamodt and Platt (1936) in Canada; and Vears and Macindoe (1935) in Australia have also presented confirmatory results.

The genetic basis for the concept of differential host reaction in oat smuts is well documented by the extensive studies of Austin and Robertson (1936), Barney (1924), Garber et al. (1929), Johnson (1933), Reed (1925, 1934, 1935, 1941, 1942), Reed and Stanton (1937, 1938) and Wakabayaski (1921) (4, 5, 27, 55, 72, 75, 76, 78, 79, 82, 83, 108). Wakabayaski demonstrated the heritability of resistance to U. kolleri in an oat hybrid of two species. It appeared that the resistance of 1 parent variety was due to 3 independent dominant factors. Barney (5), who used 12 varieties to make 31 crosses, believed that the genetics of resistance to loose smut, U. avenae, was more complex. One, 2 and 3 factor pairs for resistance were identified, depending upon the variety involved. The evidence that inheritance of resistance of U. avenae and U. kolleri was controlled by a single dominant factor pair was given for the first time by Reed in 1928. This, he concluded from a single cross between the resistant variety Black Mesdag of Avena sativa and the susceptible Avena nuda var. inermis (73). This contribution for the first time included resistance to both smut species. Garber et al. (27) also identified single dominant factor pairs for resistance to oat smut. Further studies by Reed and Stanton (1936) and Reed (1942) indicated 3 factor pairs for resistance (36, 79). Cochran et al. in 1944 reported as many as 4 factor pairs governing the inheritance of

host reaction in certain oat hybrids (12). Reed and Stanton (1938) using 1 parent that was susceptible to both loose and covered smut races, described inheritance of resistance on a 2 factor basis (83). Similarly, Johnson in a cross between Black Mesdag and Victory reported resistance to U. kolleri to be conditioned by 2 factors (55). In another cross, Marton X Colorado 37, Austin and Robertson (4) reported 2 factors conditioning resistance. From all the aforesaid evidence, it appears that resistance to loose and covered smut is conditioned by the action of 1 or more dominant genes. However, in 1 case concerning covered smut, susceptibility was reported to be dominant (82). In the same cross loose smut resistance was found to be dominant. Obviously, the host parasite interactions in oat smut diseases are very complex and not completely understood.

Among cereal smuts pathogenic specialization is common in all species. In order to conduct a successful breeding program it is essential to study inheritance of pathogenicity in oat smuts. This physiologically specialized characteristic was first studied by Faris (1924) in Ustilago hordei (20). Reed (1924) first described physiologic specialization of U. avenae (70). This phenomenon is widespread in oat smut fungi and perhaps exists in direct correlation to the host. Nicolaisen (1934) and Halisky (1954) found that pathogenicity factors may be dominant, resistant, or mixed dominant or recessive, depending upon host variety (31, 64). In this way, host-parasite interaction between the smut races and differential varieties are considered to be the basic criteria for identification and classification of pathogenic races. The heritability of pathogenicity factors in the oat smut fungi is independent of other characters and new combinations result in new physiologic races (51). The potentiality

of hybridization between races of U. avenae and U. kolleri has been cited by several workers and it may account for the origin of new physiologic races (44, 46, 48).

Because of economic considerations, a program is usually designed to improve the oat breeding program, based upon the annual surveys of races found by testing on the established differential varieties. Thus pathogenicity is the most important of race differential criteria in the oat smuts. For this reason, investigators usually emphasize this aspect of the specialization phenomenon. Consequently, the problem of physiologic specialization in oat smuts is sometimes viewed primarily as one of the pathogenic or host specialization. However, from the practical standpoint it has been observed that such programs are not always successful because of lack of stability of response in the performance of smut races from year to year on host differentials. This mainly appears to be related to the original state of impurity of the races involved.

The constancy of pathogenicity in the oat smut races has a direct correlation to the genetic purity of the original spore population. However, attempts have been made by Sampson (1925, 1929, 1933), Sampson and Western (1938), and Hallsky (1956) to bring the smut collection or races to purity level by varietal screening, by inoculations made with monosporal cultures, and by inoculating compatible paired sporidial lines from single diploid spores (32, 89, 31, 92, 93). Varietal screening has been very successful for most practical purposes and it is dependent upon the degree of selectivity of a variety or varieties. The application of the latter two techniques depends upon the degree of homozygosity of the spore from which cultures are obtained. A major problem in the studies of oat smut races arises from erratic performance in pathogenicity

on the same variety in succeeding years (100). Sometimes it takes 2 years for a race to become stable and incapable of exhibiting wide pathogenicity ranges on the same varieties. Selective influence on the host variety or varied environmental conditions in different seasons are believed to be responsible for such performance. Moreover, different seed lots of the same variety from different sources may reveal differential reaction, and sometimes it is as great as that of 2 different varieties (23). In general, oat loose smut races bear a high degree of specialization and much of the failure for their control through resistant varieties depends upon the dynamics of race populations. This problem is well illustrated by the appearance of highly specialized virulence for oat varieties that were resistant at 1 time to all the known races; Victoria and Black Mesdag oats are such examples (38, 79, 106).

The heritable nature of pathogenicity factors in oat smuts is well established and the number of factors involved and mechanism of their heritability was explained by Halisky (32) in 3 races of U. avenae, pathogenic on 6 differential varieties. He postulated that there is a mechanism of the interaction of complementary factors which governs the pathogenicity. The existence of discrete factors governing the pathogenic reactions on 2 key differential varieties, Monarch and Camas, respectively, has been substantiated with experimental evidence. It was determined by Holton and Halisky (1960), Halisky (1956) and Halisky and Holton (1956) that the existence of certain well defined races (phenotypes) of U. avenae is predicted on a multiplicity of genes for virulence, arranged in various combinations (genotypes), with the parasitic dicaryons. Based on these recent investigations, it was determined that avirulence is dominant in oat loose smut. This also indicates monogenic control of

virulence (32, 33, 51). Hybridization between virulent and avirulent races of oat smut species accounts for evolution of new races (32, 51).

Several attempts in the past have been made to key out the physiologic races of oat smuts on the basis of their pathogenic reaction on selected differential varieties. Reed (1940) described and numbered 29 races of U. avenae and 14 of U. kolleri. This was a culmination of a long series of reports on specialization in the oat smuts by him and his coworkers. About half of these races were from the United States and the remainder from other sources (77). Later Reed and Stanton (1942) and Reed, Stanton and Wilds (1947), described 2 additional races of U. avenae, both of which were able to infect Victoria, formerly resistant to all the known races (79, 85). Holton and Rodenhiser (1948) emphasized the need for correlating studies on physiologic specialization in the oat smuts with development of smut resistant varieties and proposed the adoption of a standard system for race identification in these smuts. Ten host-differential varieties were selected to maintain a standard classification system for oat smut races on the basis of susceptible and resistant reaction. From a collection of 218 specimens from oat growing regions in the United States, 15 races of U. avenae and 7 of U. kolleri were differentiated (52). Hansing (1954) described 5 additional races of U. avenae, ranging from A16 to A20. These were designated on the basis of a study in which reaction of 64 collections of U. avenae and 38 of U. kolleri on 10 previously described (39) standard differential varieties was tested. Out of these 5 new races of U. avenae, 3 were distinct Fulghum races and 2 were non-Fulghum races. A new race of U. kolleri was also characterized by its intermediate reaction on the 4 standard differentials and it was designated as K8. Fulghum and Victoria races were reported

to be more common in southern Kansas, while others were generally distributed (39). In a recent report, Luke et al. (1961) have added 2 more new races, A21 and A22 of U. avenae from their investigations on oat smut collections from the south Atlantic states (62). The reaction of these smuts was tested on oat differential varieties which are native to the South, as well as on the standard differentials used by Holton and Rodenhiser (52).

Graham (1960) attempted to study the chemical and structural components in the dwarf bunt fungus, Tilletia contraversa. He used selected reagents and procedures to separate the various wall layers of bunt teliospores to determine their chemical composition. Specific chemical tests were run on leachates and supernatant fractions to determine what materials they contained. Graham's studies involved to a very limited extent the amino acids and carbohydrates in the teliospores. This was done only by the application of a specific chemical test on the spore contents.

MATERIALS AND METHODS

In the present studies various investigations were carried out on selected oat smut races of Ustilago avenae (Pers.) Rostr. and Ustilago kolleri Wille collections. The smut material of recently described oat loose smut races, A14, A14B, A20 and A22, was obtained from Mr. S. J. Hadden, Coker's Pedigreed Seed Company, Hartsville, South Carolina. Smut race A14 originated from a collection from Victorgrain Strain 1 (C. I. 3692) and some other Victoria derivatives. Initially it was maintained on Stanton (C.I. 5116) and later on Arlington (C.I. 4657) which expressed a higher degree of susceptibility for this particular race (61). Loose smut race A20 was collected from oat varieties carrying Victoria germ plasm, especially Fultex (C.I. 3531). It was maintained on Stanton (C.I. 5116). Race A22 was collected from Fulgrain Strain 1 (C.I. 3253) in 1939. During the 1940's this race was maintained on Fulgrain Strain 3 (C.I. 3697) and since 1950 on Victorgrain (C.I. 7125).

The M5 and M11, covered smut collections, were recently collected from Mississippi and have not been designated as specific races. All smut collections have been maintained on standard susceptible varieties at Gainesville, Florida for 4 or 5 years. Therefore, they are relatively homozygous for pathogenicity factors.

Seed of the following differential oat varieties was obtained from Aberdeen, Idaho: Atlantic (C.I. 4599), AB301 (C.I. 7437), Fulghum (C.I. 708), Red Rustproof-14 (C.I. 4876), Southland (C.I. 5207),

Victorgrain 48-93 (C.I. 7125) and Wintok X Santa Fe (C.I. 7230). These varieties were used for conducting various experiments throughout the study.

Seed of 100 lines of F_2 plants from a cross between Fulgrain Strain 7 (C.I. 4389) X Fulgrain Strain 3 (C.I. 3697) were utilized for the field experiment involving studies on inheritance of host reaction. The F_1 generation of this cross was increased at Aberdeen, Idaho and the F_2 generation was grown at Gainesville, Florida.

Blakeslee's agar, malt extract agar, potato dextrose agar, potato dextrose-malt agar and water agar were used to test the rate of chlamydospore germination and other studies in culture. The ingredients of these culture media are---Blakeslee's agar: malt extract 20 g, dextrose 20 g, bacto peptone 1 g, agar 20 g and water 1 liter; malt extract agar: malt extract 20 g, peptone 1 g, agar 20 g and water 1 liter; potato dextrose agar (difco): potato infusion from 200 g, bacto dextrose 20 g, bacto agar 15 g and water 1 liter; potato dextrose-malt extract agar: potato dextrose agar (difco) 39 g, malt extract 20 g and water 1 liter; water agar: bacto agar 20 g and water 1 liter; potato sucrose agar: potato infusion from 200 g, sucrose 20 g, bacto agar 20 g and water 1 liter. Ingredients of each culture medium were autoclaved at 15 pounds pressure for 20 minutes.

Chlamydospores were surface sterilized by immersing in 0.1% copper sulfate, washed in sterilized distilled water for 10 minutes and placed on the culture media. The rate of germination was determined by allowing spores to germinate on agar drops on the surface of microscope slides within petri plates. A drop of melted agar medium was placed on the

surface of a sterilized slide and allowed to solidify. A small drop from the spore suspension was transferred to the agar drop, which was incubated at 22 - 24° C. The percentage of spore germination was determined at 24, 48, 72 and 96 hour intervals.

In another experiment, effect of 5 different temperatures and 4 culture media on spore germination were determined. A14 and A22 loose smut races, as well as M5 and M11 covered smut collections, were used in this study. Spores were germinated on Blakeslee's agar, malt extract agar, potato dextrose agar and potato sucrose agar, at 20°, 24°, 28°, 32°, and 36° C. The procedures for inoculation process were the same as described above. Moist chambers with each smut culture were placed inside the incubators, which were set at appropriate temperatures and the data were collected after a 96 hour period. Only the chlamydospores which produced well developed promycelial cells were recorded at specified time intervals in all the tests pertaining to spore germination.

In addition, growth rate of 4 smut cultures was determined on Blakeslee's agar, malt extract agar, potato dextrose agar, potato dextrose-malt agar and potato sucrose agar. These tests were conducted in 9 cm petri plates, using 4 replications for each smut culture. The inoculum was applied by transferring a 1 mm disc from vigorously growing stock cultures of each smut. Since growth is correlated to the diameter of each smut colony, the growth rate on solid media was estimated by measuring in millimeters the diameter of the smut colony. All the cultures were allowed to grow for 3 weeks at 22° - 24° C.

Growth rates of the aforementioned cultures were also determined on liquid media by growing them in 250 ml Erlenmeyer flasks which contained

35 ml of the above mentioned liquids to which no agar was added. Three replications for each treatment were used. A 1 mm disc of each culture (A14, A22, H5 and H11) was transferred to the flasks. The cultures were grown at 22° - 24° C and flasks were subjected to constant agitation by placing them on a shaker. The period allowed for growth in liquid media was 3 weeks. After this interval the cultures were filtered through a Buchner funnel. Harvested fungal mats were dried in a hot air oven at 95° C for 12 hours and the dry weights were determined by weighing on an analytical balance.

Two experiments were conducted to test the effect of spore age and culture media on the degree of smut infection on selected oat varieties. Atlantic and Victorgrain oat varieties were used to test the reaction of interracial hybrids of loose smut races A14 and A22. In the initial experiment hybrid spores were grown on potato dextrose agar, potato dextrose-Victorgrain extract agar, and Victorgrain extract water agar. The latter two may be regarded as specialized media. From this experiment it was found that potato dextrose-Victorgrain extract medium maintained high pathogenicity of inoculum as compared to potato dextrose and Victorgrain extract water agar. The use of the latter 2 media was eliminated and only potato dextrose-Victorgrain extract medium was used in the second experiment. Ingredients of potato dextrose-Victorgrain extract agar consist of: potato dextrose agar (difco) 39 g, leaf extract from 40 g Victorgrain oat leaves and 1 liter of water. Victorgrain extract water agar includes extract from 40 g Victorgrain leaves, 25 g bacto agar and 1 liter of water. For both specialized media, the Victorgrain leaves obtained from 4 week old seedlings were extracted with a measured quantity of hot water in a Waring blendor for 10 minutes. Leaf extract was filtered

through cheese cloth, other ingredients of the respective media were added and the final volume was made up to 1 liter by adding distilled water. These were heated to boiling point and sterilized in an autoclave.

For hybridizing races A14 and A22 of U. avenae, single chlamydospores of each pathogen were isolated with the aid of the Fonbrune pneumatic micromanipulator. These chlamydospores were allowed to germinate on agar drops of the 3 aforementioned agar media within moist chambers. The single isolated chlamydospores of each smut were allowed to grow for about 24 hours till they produced the complete set of 4 haploid sporidia. Immediately after this period the agar drop with 4 primary sporidia was transferred to the respective agar plates. In this manner monochlamydospore transfers of both loose smut races were made in separate agar plates on each of the 3 kinds of media. These developed into 4 haploid sporidial colonies within 1 week. Each monosporidial colony was isolated singly in fresh agar plates and allowed to grow till they attained a considerable size after multiplication by vegetative budding.

Thus, complete sporidial sets from single chlamydospores of races A14 and A22 were maintained on potato dextrose agar, potato dextrose-Victorgrain extract agar and Victorgrain extract water agar. Crosses were made on 3 agar media by transferring the haploid sporidia of races A14 and A22 with a sterilized platinum loop in all the possible combinations. The compatibility among sexually mating types was visible by development of white mycelial "Suchafaden" as described by Bauch (6). F_1 diploid spores developed in culture after a period of 2 weeks. This was confirmed by microscopic examination of cultures. Several F_1

interracial hybrid spores were transferred to agar plates on the 3 media and allowed to grow till they developed the F_2 population by self fertilization. Since the avirulence in oat loose smuts is dominant, no smut infection can be predicted by F_1 diploid spores. Therefore, for this reason only F_2 interracial hybrids were used for inoculation purpose in this study.

Efforts were made to determine the effect of age of F_2 hybrids on the degree of smut infection. For this purpose 10, 20 and 30 day old F_2 spores were used as inoculum.

Allison's (2) partial vacuum method for seed inoculation was used in all experiments that were conducted in the greenhouse and growth chamber. The non-dehulled seed of each oat variety was immersed in sterilized distilled water suspension of inoculum in screw cap vials. Vials with tops screwed loosely were placed in a vacuum desiccator and subjected to partial vacuum for 20 minutes. The vacuum was released abruptly to allow the spores to enter the evacuated space between the lemma and palea. After settling, the water suspension was drained out and inoculated seed was spread and dried on paper towels under cool, dry conditions. The dried seeds were planted (10 seeds/pot) in 6-inch pots. Four replications were used for each treatment. Prior to the planting, soil used in pots was treated with methyl bromide.

Experiments were conducted to determine the effect of loose and covered smut infection on rate of growth of certain host varieties. The pathogenic influence of race A14 of U. avenae and collection M5 of U. kolleri was observed on the Fulghum oat variety. The reaction of race A22 of U. avenae on the rate of growth of Red Rustproof 14 and Victorgrain oat varieties was also determined. The technique for

inoculating the seeds with respective smut spores was the same as described previously. Four replications (10 seeds/pot) were used; however, the data were taken only from 8 plants of each replication. The rate of growth was evaluated by dry weight method. Oat clippings of each treatment were collected for 8, 16, 24, 32 and 40 day growth periods. Clippings were dried in paper bags within a hot-air oven at 95° C for 24 hours and dry weights were determined. Uninoculated seeds of each oat variety were also planted; their dry weights were determined for same growth intervals as in the smut treated plants. These served as uninoculated checks. Another series of inoculated seeds were planted which were allowed to mature and develop smutted panicles. This test consisted of 10 pots (10 seeds/pot); each was planted with Fulghum seeds after they were inoculated with A14 and M5 smuts. Similarly, 10 pots each of Red Rustproof 14 and Victorgrain oats inoculated with A22 were planted under identical conditions in a growth chamber in which temperature was maintained at 72 - 74° F. All the pots with inoculated check plants were fertilized with Hygro when they were 5 weeks old. The smut percentage in each case was determined on a plant basis.

The inheritance of host reaction to loose smut races A20 and A22 was determined using 100, F_2 plant families of Fulgrain Strain 7 X Fulgrain Strain 3. Strain 7 is susceptible to A20 and Fulgrain Strain 3 is susceptible to A22. Two replications were used for each treatment, and 2 g of seed per treatment were weighed from each of the 100 F_2 lines. Allison's (2) partial vacuum technique was used to inoculate the seed used in this experiment. Following inoculation, seeds were spread on large blotting sheets for 24 hours under cool conditions. After

inoculations seeds were planted in the field in rows 5 feet long. Seeds of the parent varieties were also inoculated with races A20 and A22 and planted as checks. The check plots consisted of 12 replications of each parental variety. Percentage of infection was calculated for each row on a plant basis (number of smutted plants ÷ total number per row X 100). The test in which race A22 was used was repeated; however, only 1 test was obtained with race A20.

CULTURE STUDIES

Both species of oat smuts are facultative parasites and can be grown on culture media if they are supplied with adequate nutritional sources in the correct proportions. In oat smuts it is recognized that requirements and responses for growth in culture media vary somewhat, not only between 2 species, but also within each species. All smut fungi need some source of carbon, nitrogen, as well as limited mineral nutrition. In the past, a variety of culture media have been developed for specific kinds of smut growth, e. g. sporidial or mycelial. Certain sporidia producing races of U. avenae and U. kolleri, after subsequent transfers, were made to produce mycelium by decreasing the amount of moisture in the culture media (87). Since various races and species of oat smuts require specific cultural conditions it was necessary to develop cultural techniques for the experimental material to be used.

The objective of the culture studies was to determine the effect of media and temperature on the rate of chlamydospore germination. Rate of growth on liquid and solid media was also determined. The initial experiments were carried out with races A14 and A22 of U. avenae as well as collections M5 and M11 of U. kolleri.

In another study the rate of growth of 4 smut cultures was determined on certain solid and liquid culture media.

Details for the experimental procedures pertaining to culture studies are found in materials and methods.

The effect of culture media on chlamydospore germination. - Results (Table 1) indicate that after 96 hours the germination percentage among loose smut races, A14 and A22, as compared to collections M5 and M11, was highest on all the 5 culture media. Moreover, the 2 loose smut races exhibited a material difference between the 72 and 96 hour test periods. This trend was not observed for the 2 covered smuts. In general, the loose smut races germinate more slowly on culture media than do the covered smut collections.

When the data in Table 1 were studied, 2 contrasting trends were observed among the loose smut races, i.e. race A14 germinated well (93%) on Blakeslee's medium while race A22 germinated poorly (below 10%). On the other hand, race A22 expressed the highest rate (94%) of germination on water agar; whereas race A14 expressed the lowest percentage (72%) of germination on this agar. No contrasting germination response was observed for the covered smut cultures. Collection M5 germinated equally well on all 5 media; however, collection M11 expressed a differential germination response to the 5 media. For example, M11 germinated best (72%) on potato dextrose agar and poorest (54%) on water agar.

Effect of temperature and nutrient media on spore germination. - Results (Table 2) show that the highest spore germination percentage of the 4 smut cultures on all the 4 media was observed at 24° - 32° C. In general 20° and 36° C caused a retardation of germination of all cultures on all media. Hence, 24° - 32° C can be rated as the optimum temperature range for loose smut races A14 and A22 as well as collection M5 and M11 of covered smut. No strong interaction between the temperature and media treatments was observed. Both loose smut races

Table 1. Percentage of chlamydospore[a] germination of races A14 and A22 of U. avenae and collections M5 and M11 of U. kolleri at various time intervals on selected culture media

Culture Media	Race A14				Race A22				Collection M5				Collection M11			
	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.
Blakeslee's agar	70	82	91	93	0	0	0	trace[b]	80	85	92	92	47	54	60	60
Malt extract agar	58	62	69	81	trace[b]	30	68	70	80	81	85	86	56	59	59	59
Potato dextrose agar	80	86	95	98	68	73	75	76	83	86	87	89	61	64	72	72
Potato dextrose malt extract agar	62	64	73	84	25	30	35	40	80	84	88	90	66	67	70	70
Water agar	54	58	65	72	81	90	92	94	71	74	80	81	46	54	54	54

[a] Each germination percentage was calculated from a total of 250 spores.

[b] Below 10% germination.

germinated best on potato dextrose agar at 28° C; whereas the covered smut collections exhibited the highest percentage of germination on this media at 32° C.

Rate of growth on solid media. - The objective of this experiment was to determine the rate of growth of 2 loose smut races and 2 collections of covered smut on Blakeslee's agar, malt extract agar, potato dextrose agar, potato dextrose-malt extract agar and potato sucrose agar. Each isolate was transferred from slants into 9 cm petri plates which were previously poured with respective agar media. Measurement (diameter of colony in mm) of the growing cultures was used as the criterion for rating growth. Averages of 4 replications of each treatment were evaluated at the end of a 21 day growth period.

Results of this study (Table 3) indicate that races A14 and A22 of U. avenae and collection M5 of U. kolleri grow most vigorously on potato dextrose-malt extract agar. Collection M11 of covered smut is apparently a slow growing organism, exhibiting best growth on Blakeslee's agar. Race A14 exhibited the highest growth rate on all media, except for M5 on potato sucrose agar.

On the basis of experimental evidence, general superiority of potato dextrose-malt extract agar for the growth rate of all smut cultures, except M11 was observed. Apparently, malt extract in combination with potato dextrose stimulates growth. Similar results were obtained by Sartoris (94). Potato dextrose-malt extract agar was superior to potato dextrose and malt extract used singly. Of the media used, dextrose, malt extract and potato in combination supplied the proper balanced nutrition for optimum growth.

Table 2. Percentage of chlamydospore germination[a] of races A14 and A22 of U. avenae and collections M5 and M11 of U. kolleri grown on 4 culture media for 96 hours, at various temperatures

Culture media	Race A14					Race A22					Collection M5					Collection M11				
	20°C	24°C	28°C	32°C	36°C	20°C	24°C	28°C	32°C	36°C	20°C	24°C	28°C	32°C	36°C	20°C	24°C	28°C	32°C	36°C
Blakeslee's agar	82	90	92	94	75	0	0	5	5	0	31	33	36	68	30	26	30	38	65	34
Potato dextrose agar	87	95	100	96	72	72	80	83	73	48	40	48	69	91	65	42	50	70	75	40
Malt extract agar	85	92	90	83	79	50	70	75	68	50	22	26	24	79	40	20	28	32	60	26
Potato sucrose agar	20	22	30	25	15	10	25	32	28	11	15	27	30	35	22	18	22	25	32	12

[a] Each germination percentage was calculated from a total of 250 spores.

Rate of growth on liquid media. - The growth rate of A14, A22, M5 and M11 was estimated on Blakeslee's, malt extract, potato dextrose, potato dextrose-malt extract and potato sucrose liquid media. Ingredients of 5 liquid media are listed in materials and methods. Cultures were grown for 3 week period in Erlenmeyer flasks; 3 replications for each treatment were used. Constant agitation was provided for higher aeration of cultures by placing the flasks on an Eberback shaker. Criterion for measuring growth was dry weight determination as described in the materials and methods.

Results (Table 4) indicate that all 4 smut cultures grew most rapidly on Blakeslee's medium. Among loose smut races, A14 grew more vigorously than race A22 on Blakeslee's and potato dextrose-malt extract media; however, there was no material difference in the growth rate of these 2 races on the other media used. Among covered smut collections, M5 showed relatively higher growth rate on Blakeslee's, malt-extract and potato dextrose-malt extract media, while M11 expressed better growth on potato dextrose and potato sucrose media.

The relatively superior growth of M5 on Blakeslee's, malt extract and potato dextrose-malt extract liquid media indicates that this culture requires some ingredient of malt extract for optimum growth. The enhancement of growth was not observed for collection M11, nor was this trend observed for either of the loose smut races. It was of interest to note that the growth rates on Blakeslee's medium were materially greater than those obtained in the other media. Excessive growth on Blakeslee's medium is not due to malt extract, because 2 other media contain this ingredient. Therefore, this pronounced growth must

Table 3. Rate of growth (diameter measurements in millimeters) of races A14 and A22 of U. avenae and collections M5 and M11 of U. kolleri on 5 culture media for a 3 week period

Smut	Average[a] growth in millimeters				
	Blakeslee's agar	Malt extract agar	Potato dextrose agar	Potato dextrose-malt-extract agar	Potato sucrose agar
Race A14	5.1	4.1	5.6	7.1	2.2
Race A22	1.9	3.1	4.3	6.7	2.0
Collection M5	3.1	2.3	4.0	6.4	3.2
Collection M11	2.7	1.1	2.1	1.7	2.0

[a] Each average consists of 4 replications of each treatment.

Table 4. Rate of growth (dry weights in milligrams) of races A14 and A22 of U. avenae and collections M5 and M11 of U. kolleri on 5 liquid (shake) media for a 3 week period

Smut	Average growth[a] in milligrams				
	Blakeslee's medium	Malt extract medium	Potato dextrose medium	Potato dextrose-malt extract medium	Potato sucrose medium
Race A14	1302	118	247	348	300
Race A22	436	105	241	250	291
Collection M5	1003	221	124	177	155
Collection M11	653	115	197	160	187

[a] Each average consists of three replications of each treatment.

be due to a balance of nutritional requirements for growth which are supplied by the Blakeslee's medium.

One of the objectives of the growth experiments was to study the effect of nutrition on the growth response of wide and narrow host range smut collections. In this respect, race A14 and collection M1 are wide range pathogens and race A22 and collection M5 are narrow range pathogens. Narrow range types may require a more specific nutritional environment than the wide range types. From the data (Table 3) there appears to be no relation between pathogenicity range and nutritional requirements in vitro; therefore, there appears to be no correlation between nutritional requirements and host range. While no correlation between nutritional requirements for growth and the nutritional requirements for pathogenicity was found, it is possible that the range of media used may have been insufficient to establish a correlation.

EFFECT OF SPORE AGE AND CULTURE MEDIA ON DEGREE OF SMUT INFECTION

In the past, various races of oat smuts have been hybridized on culture media and their pathogenicity has been studied on oat varieties. The problem of loss in pathogenicity by spores produced on culture media was not reported by early workers. However, in the present studies, results from an exploratory test indicated that culture medium and/or spore age affected the degree of smut infection. Therefore, it seemed desirable to conduct experiments to determine the effect of culture media and spore age on the pathogenicity of F_2 progeny of an interracial hybrid. It has been observed that there is a variable degree of smut infection incited by hybrid and selfed spores. Therefore, to evaluate the data, it was considered necessary to use interracial hybrids as well as selfed spores (monochlamydospores) in the present study.

Two experiments were carried out. Initially, A1^4 X A22, F_2 hybrids were grown for 10 and 20 days on potato dextrose agar, potato dextrose-Victorgrain extract agar and Victorgrain extract-water agar. The degree of smut infection was tested on Atlantic and Victorgrain oat varieties. Atlantic is susceptible to race A1^4 and Victorgrain is susceptible to race A22. The reaction of selfed spores of each race was also tested on these oat varieties.

From the first experiment, results showed that spores grown on potato dextrose-Victorgrain extract-agar incited higher percentage infection than those grown on the other media. Therefore, potato

dextrose-Victorgrain extract agar was the only medium used in the second experiment. The second experiment was similar to one described in the preceding section, except that spores were grown for 10, 20 and 30 day periods. Atlantic and Victorgrain varieties were used. Procedure for producing the hybrid spores on various culture media are found in the materials and methods. Selfed spores of races A14 and A22 were produced by isolating single chlamydospores with the aid of a Fonbrune micromanipulator. Diploid spores developed in 7 - 10 days after the mating of haploid sporidia. Ten, 20 and 30-day-old diploid F_2 spores were used for inoculation. Allison's (2) partial vacuum method was used for seed inoculation. Both experiments were conducted in a growth chamber which maintained temperatures between 72° - 74° F.

Results (Tables 5 and 6) indicate that both 10 and 20-day-old hybrid as well as selfed spores grown on potato-dextrose-Victorgrain extract agar cause the highest degree of infection. Moreover, 20-day-old spores incited a higher percentage of infection than did 10-day-old spores, regardless of the medium on which they were produced.

Results from the second experiment (Table 7) indicate that both, hybrid and selfed spores which were 20 days old produced the highest percentage of infection. Ten-day-old spores were more infectious than 30-day-old spores.

Spores produced on potato dextrose-Victorgrain medium were materially more infectious than those produced on potato dextrose agar or Victorgrain extract-water agar. Since the latter 2 contained all the ingredients of the mixture, increased infection cannot be attributed to any single component (Victorgrain extract, potato dextrose) of the media used.

Table 5. Percentage of smut infection[a] on Atlantic incited by interracial hybrid and selfed spores of U. avenae which were grown on 3 different culture media for 10 and 20 days

Age of spores in days	potato dextrose agar			potato dextrose-Victorgrain extract agar			Victorgrain extract-water agar		
		selfed			selfed			selfed	
	A14 x A22	A14	A22	A14 x A22	A14	A22	A14 x A22	A14	A22
10	61	50	0	83	67	0	65	33	0
20	89	67	0	100	94	0	78	72	0

[a] Each percentage consists of 4 replications of 6 plants.

Table 6. Percentage of smut infection[a] on Victorgrain incited by interracial hybrid and selfed spores of U. avenae which were grown on 3 different culture media for 10 and 20 days

Age of spores in days	potato dextrose agar			potato dextrose-Victorgrain extract agar			Victorgrain extract-water agar		
		selfed			selfed			selfed	
	A1 4 X A22	A1 4	A22	A1 4 X A22	A1 4	A22	A1 4 X A22	A1 4	A22
10	28	0	30	61	0	33	39	0	15
20	83	0	61	89	0	67	61	0	45

[a] Each percentage consists of 4 replications of 6 plants.

Table 7. Percentage of smut infection[a] on Atlantic and Victorgrain incited by interracial hybrid and selfed spores of U. avenae which were grown on potato dextrose-Victorgrain extract agar for 10, 20 and 30 days

Age of spores in days	Atlantic			Victorgrain		
		selfed			selfed	
	A14 x A22	A14	A22	A14 x A22	A14	A22
10	84	80	0	73	0	53
20	93	87	0	85	0	62
30	55	50	0	26	0	20

[a] Each percentage consists of 4 replications of 6 plants each.

Therefore, the increased pathogenicity of spores grown on potato dextrose-Victorgrain extract must be due to the balance of nutritional factors of this medium.

The 20-day-old spores were more infectious than 10 or 30-day-old spores. Apparently, immaturity accounts for the low infection caused by 10-day-old spores, whereas lack of viability due to staling products was responsible for the lowering of infection of 30-day-old spores.

EFFECT OF SMUT INFECTION ON THE RATE OF GROWTH OF CERTAIN OAT VARIETIES

Several workers have studied the effect of smut infection on host development (germination, height, diameter of culm, and yield) (54, 110). However, little attention has been given to the effect of infection on growth of the host. Therefore, the present studies were designed to determine the effect of infection by U. avenae and U. kolleri on the rate of growth of selected oat varieties.

The effect of race A14 of U. avenae and collection M5 of U. kolleri on the rate of growth of the Fulghum oat variety was observed. The effect of race A22 on the growth rate of Red Rustproof-14 and Victorgrain oat varieties was also studied. All oat varieties used are susceptible to the respective smuts with which inoculations were made. Allison's (2) partial vacuum method for seed inoculation was used. Inoculated seeds were planted (10 seeds per 8-inch pot) and placed in a growth chamber which maintained the temperature between 72° and 76° F. The rate of growth was evaluated by the dry weight method. Oat clippings of each treatment were collected for 8, 16, 24, 32, and 40 day periods following inoculation, and dried in paper bags within a hot air oven at 95° C for 24 hours. The clippings were taken from 4 replications of each treatment. Uninoculated seeds were also planted in the same manner; these served as uninoculated checks. Another series of inoculated seeds were planted and were allowed to mature and produce smutted panicles. This part of the experiment was necessary to estimate the percentage infection of the treated plants.

No appreciable difference in the growth rate of Fulghum as a result of infection with A14 and M5 was noted after 8 and 16 day growth periods. However, after the 24 day period some difference in the dry weights of plants, inoculated with race A14 of U. avenae and collection M5 of U. kolleri occurred. The rate of growth of host plants to some extent was reduced as a result of the pathogenic influence of A14. The same trend continued for the remainder of the test period. On the contrary, plants infected with M5 exhibited an increase in growth as compared to the uninoculated checks. The variation in rate of growth of Fulghum due to pathogenic influence of A14 and M5 was significant at 5% level (Table 8).

The lack of influence on growth of the host during early stages of development might be explained as due to a retardation of growth of both pathogens during the incubation period. Perhaps, A14 and M5 produce some metabolite during their infection phase on Fulghum which interacts to express a contrasting reaction from the standpoint of altering the growth rate. This conclusion appears valid since there was no significant difference in the infection (A14 - 70%; M5 - 60%) incited by the 2 pathogens on the host.

The experiment in which Red Rustproof-14 and Victorgrain oat varieties were inoculated with race A22, indicates that this pathogen incites varied influence on the growth rate of these hosts (Table 9). For example, race A22 decreased the growth rate of Red Rustproof-14 but caused a marked increase in the growth rate of Victorgrain. The growth changes incited by this pathogen were significant at the 1% level (Table 9). Approximately 90% smut infection was observed for Victorgrain and 30% for Red Rustproof-14. No distinction between the

Table 8. Dry weights of inoculated and healthy Fulghum oats grown for 8, 16, 24, 32, and 40 days following inoculation with race A14 of loose smut and collection M5 of covered smut

Days	Mean dry weight[a] in milligrams		
	Race A14	Collection M5	Uninoculated checks
8	150	180	175
16	182	230	219
24	450	600	502
32	856	1300	1100
40	1250	1700	1500

Statistical Analysis

Source	D/F	SS	Mean Square	F
Total	59			
Culture	2	0.513	0.256	1.96
Time	4	15.637	3.909	29.91**
Culture X Time	8	0.247	0.031	2.37*
Replication	3	0.030	0.010	0.76
Error	42	0.549	0.013	

[a] Each mean is made up of 4 replications, 8 plants each.
** Significance at 1% level.
* Significance at 5% level.

Table 9. Dry weights of inoculated and healthy Red Rustproof-14 and Victorgrain oats grown for 8, 16, 24, 32, and 40 days following inoculation with race A22 of loose smut

Days	Mean dry weights[a] in milligrams			
	Red Rustproof-14 inoculated	Red Rustproof-14 uninoculated	Victorgrain inoculated	Victorgrain uninoculated
8	120	140	110	110
16	192	224	220	148
24	402	480	480	412
32	850	910	1600	1110
40	1200	1340	1832	1802

Statistical Analysis

Source	D/F	SS	Mean Square	F
Total	79			
Variety	1	0.74	0.74	140.00**
Time	4	25.00	6.25	1250.00**
Infection Class	1	0.01	0.01	2.00
Variety X Time	4	0.52	0.13	26.00**
Variety X Infection Class	1	0.21	0.21	42.00**
Time X Infection Class	4	0.21	0.05	10.00**

Table 9. Continued

Statistical Analysis

Source	D/F	SS	Mean Square	F
Variety X Time X Infection Class	4	1.91	0.48	96.00**
Replication	3	0.12	0.04	8.00**
Error	57	0.29	0.005	

[a] Each mean is made up of 4 replications, 8 plants each.
** Significance at 1% level.

smutted and healthy clippings of Victorgrain was observed for the first 8 day growth period; however, inoculated plants began to show some increase in the vegetative growth for 16, 24, 32, and 40 day intervals. The highest increase was attained when plants were 32 days old. Little difference between healthy and diseased clippings of Victorgrain was observed when they were 40 days old.

It is interesting to note that race A22 of U. avenae which is highly pathogenic on Victorgrain (90% smut) appears to enhance the growth rate; whereas a low order of infection on Red Rustproof-14 (30% smut), appears to retard growth of the host. Other workers (54, 98) have shown that smut infection retards host development; therefore the reduction of growth of Red Rustproof-14 and Fulghum by races, A22 and A14, respectively, is not surprising. On the other hand, the highly significant increase in growth of Victorgrain by race A22 (Table 9) may be similar to the effect of Gibberella fujikuroi on rice plants (99). That is, race A22 either excretes an endogenous growth regulator or the host-parasite interaction results in the release of a growth regulator. This may function in a similar manner as the gibberellins (10) which appear to exert their physiological effects by removing inhibitors in cell enlargement. Moreover, the growth changes due to smut infection appear to be differentially specific, whereas the effects of G. fujikuroi express a nonspecific reaction.

INHERITANCE OF HOST REACTION TO SMUT INFECTION

Extensive studies have been made by many workers on inheritance of host reaction to U. avenae and U. kolleri (23, 50). Such investigations involved a great number of oat varieties consisting of widely different genetic make up. Information from these studies has been useful in the breeding of smut resistant varieties in oat growing areas where loose and covered smuts of oats are severe. Resistance to certain oat smuts is controlled by a variable number (one to several) of genetic factors (12, 36, 73, 79, 83). The number that can be identified depends upon the variety, source of resistance, and pathogenic races involved. With one exception in the covered smuts (82) all reports indicated that resistance to oat smut pathogens was controlled by dominant gene action. The primary objective of the present study was to determine the inheritance of host reaction to wide and narrow host-range pathogens. Host material and its reaction to the pathogens used is found in the materials and methods.

In the present studies, inheritance of host reaction of F_3 plants derived from F_2 plant families to 2 recently described (62) races of U. avenae was determined. One hundred F_2 plant families of Fulgrain Strain 7 X Fulgrain Strain 3 and races, A20, A22 were used. Two g of seed per treatment were weighed from each of the 100 F_2 lines and 2 replications were used for each treatment. Seeds were inoculated by Allison's (2) partial vacuum technique. The inoculated seeds were planted in 5 feet rows in field plots. Seeds of each parent variety

(Fulgrain Strain 7 and Fulgrain Strain 3) were also inoculated with races A20 and A22 and planted as checks. The check plots included 12 replications of each parental variety. Infection percentage was calculated for each row on a plant basis (number of smutted plants per row ÷ total number per row X 100). The test in which race A22 was used was conducted twice; however, only one test was carried out with race A20.

The 1961 test in which 100 F_2 plant families of Fulgrain Strain 7 X Fulgrain Strain 3 were inoculated with race A22, 1 - 75% smut infection was observed in 72 families whereas 28 families did not show any infection. In the 1962 test, 1 - 92% infection was obtained among 75 families and 25 families were smut free. Among the 100 F_2 families of the same oat hybrid which were inoculated with race A20, 76 families were found to be healthy and only 24 showed smut infection, ranging from 1 - 74%. Among the check plots which were planted with inoculated parental varieties, race A20 infected 70% of the plants of Fulgrain Strain 7 and no infection was observed on Fulgrain Strain 3. Race A22 infected 95% of the Fulgrain Strain 3 plants and revealed no infection on Fulgrain 7.

The experimental evidence indicates that susceptibility is dominant over resistance when reaction to race A22 is considered. This constitutes the first report that resistance to loose smut is controlled by recessive gene action. On the contrary, the reaction of plants from the same hybrid to race A20 indicates that resistance is dominant and susceptibility is recessive. The statistical analysis of data indicate monogenic inheritance of host reaction to races A20 and A22 (A20, $X^2 = 0.053$, $P = 83.3\%$; A22, $X^2 = 0.12$, $P = 73.3\%$).

Independence of the loci was explored. Although the data did not fit the 9:3:3:1 independence ratio ($\chi^2 = 17.32$; $P = 11\%$), linkage tests indicated that no improvement in fit was possible. Therefore, classification difficulties were considered to be responsible for the deviation from independence and the 2 loci were assumed to be independent or on separate chromosomes.

CHEMOTAXONOMIC STUDIES WITH OAT SMUT SPECIES

Many reports have been made concerning physiologic specialization in oat and other cereal smuts. Usually, these investigations involved cultural and pathogenic specialization. Such criteria have been used to identify individual races and to establish a systematic classification of these fungi. Biochemical data used as a criterion of classification should supplement systematic studies. This approach may lead to the development of more critical techniques of classification. Hence, in the present studies, a series of experiments were carried out to determine inter or intraspecific biochemical differences among loose and covered smuts. (Chemotaxonomy as used in this thesis denotes chemical differences or similarities within races of both species or between races of both species). Chemotaxonomic relationships were studied using narrow and wide range pathogens. These smuts were used on the assumption that pathogenicity range may be related to nutritional requirements which in turn may be reflected by their biochemical constitution.

Chemotaxonomic studies were conducted on races $A1^4$, $A1^{4B}$, A22 and collection 4 of U. avenae, and collections M5 and M11 of U. kolleri. The source of $A1^4$, $A1^{4B}$, A22, M5 and M11 smuts was given earlier. Collection 4 was originally collected from Florland at Quincy, Florida in 1959. In 1960 it was collected from Red Rustproof-14 and in 1961 from C.I. 7230. For the present study smut spore material was collected from Victorgrain and C.I. 7230 during spring of 1962.

Chemotaxonomic studies were carried out using a paper chromatographic technique to determine the free and bound amino acids in chlamydospores of the above mentioned smuts. Studies were further extended to determine the reducing sugar content of chlamydospores in A14, A14B, A22, M5 and M11; collection 4 of U. avenae from C.I. 7230 and Victorgrain were not included for analysis of the reducing sugars. To remove traces of host tissue and smut balls, the spore mass of each smut was strained through a No. 40 mesh screen. Amino acids and reducing sugars were analyzed from 0.5 g of chlamydospores of each smut.

Preparation of free amino acid fractions. - Chlamydospores of each smut were transferred to 80% boiling ethanol. The spore-ethanol suspension was immediately homogenized in a Servall omnimixer for 5 minutes. The homogenized spore suspensions were boiled on a hot plate for 20 minutes in order to attain complete extractions. The material was centrifuged at 20,000 x gravity for 10 minutes. The supernatant was poured in porcelain dishes and reduced to dryness in a cold air stream. Ten percent isopropanol was added to make the ultimate volume of each sample up to 1 ml; extracts were stored in small glass vials in the refrigerator.

Preparation of bound amino acid fractions. - The spore residue obtained after centrifugation from the above treatment was used to prepare the protein amino acid samples. The residual material was dried to remove the traces of ethanol. Ten ml of 6 N hydrochloric acid was added to the chlamydospore residue of each smut in 50 ml Erlenmeyer flasks. The mouths of flasks were capped with round marbles and these were placed on a hot plate for 12 hours. After the protein hydrolysis was completed the hydrolysate was centrifuged

at 20,000 x gravity for 10 minutes, and desalted on 0.5 g of Dowex - 50 x 8 ion exchange resin. The sugars and anions were washed through and amino acids were eluted with 25 ml of 2N ammonium hydroxide. The eluate was evaporated to dryness and extracts were rehydrated with 1 ml of 10% isopropanol.

Preparation of reducing sugar fractions. - Five tenths of a g of chlamydospores of A14, A14B, A22, M5 and M11 smut cultures were each homogenized in 25 ml of 80% ethanol and centrifuged in the same way as for free amino acid samples. The excess ethanol was evaporated and the volume was reduced to 10 ml. Each sample was deproteinized by treating with 5% trichloroacetic acid. These were again centrifuged at 20,000 x gravity for 10 minutes. The supernatant was poured into porcelain cups and evaporated to dryness. The final volume was adjusted to 1 ml by adding 10% isopropanol.

Chromatographic procedure. - For both kinds of amino acids and reducing sugars, descending chromatographic techniques were used (101). Whatman No. 3 MM paper was used for all amino acid analyses. The technique was carried out using a 2 dimensional (butanol, acetic acid, water and phenol, ammonia, water) solvent system. These tests were conducted in standard chromatocabs which were maintained at 22° - 24° C. Twenty-five to 30 lambda of the amino acid samples were spotted on each paper. This was accomplished by applying 10 lambda samples and allowing each sample to dry before the following application. The composition of solvents used for amino acid analyses have been previously described (97). After the chromatograms were run with butanol acetic acid, they were dried for 4 hours in a well ventilated hood and the phenol-solvent was run in a separate chromatocab. The second solvent was also

evaporated from the papers in a well ventilated hood for 6 to 8 hours. Chromatograms were dipped in 0.25% ninhydrin in acetone and dried as described above. Attempts to identify various amino acids were made by calculating the Rf values. Identification of specific amino acids was further substantiated by spotting known amino acids along with the extract sample. In addition, special solvent systems were also used for locating particular amino acids, e.g. butanol pyridine and methanol pyridine for differentiating histidine and methionine in the loose smut samples; moreover, sulfanilic acid developer was used to differentiate these amino acids. Four 2-dimensional chromatograms were run from each smut sample.

Sugars. - The reducing sugars from 5 smut samples were separated on Whatman No. 1, 22" x 1 1/2" chromatogram strips. Isopropanol-water (160:40) and isopropanol-butanol-water (140:20:40) solvent systems were most satisfactory for separating various reducing sugars present in the samples. An isopropanol-pyridine-water (120:40:40) solvent system was found to be unsatisfactory for sugar analysis. The chromatograms were developed by using several color reagents to locate various sugars. These were: silver nitrate reagent (silver nitrate saturated solution, in water - 0.1 vol, acetone - 20 vol, sodium hydroxide, 0.5% in 80% ethanol), aniline-diphenylamine (aniline, 1% - 1 ml, diphenylamine, 1% in acetone - 10 vol, phosphoric acid, 85% - 1 vol), aniline phthalate (aniline, 930 mg, phthalic acid 1.6 g, 100 ml water saturated n-butanol) (8, 97). Silver nitrate reagent proved to be the best developer for reducing sugars; hence its use was continued and others were eliminated.

The chromatogram strips were first dipped through silver nitrate solution and the acetone was blown off. When dry, they were dipped

through the sodium hydroxide solution and the solvent was again blown off in a well ventilated hood. Spots began to appear at once for more reactive sugars; all others were visible in 10 minutes at room temperature, giving dark brown spots on a yellowish background. Ammonia (2N) was used to bleach the background. Identification of various reducing sugars from the smut samples were made by comparing with known sugars.

Free amino acids. - Results (Table 10) of the experiment concerning analysis of free amino acids indicate that 3 races, A14, A14B and A22 of U. avenae contain histidine and methionine. These 2 amino acids were absent in collections M5 and M11 of U. kolleri. Considering the interracial differences among the 3 loose smut races, it is evident that race A14B contains the largest number of free amino acids. For example, tyrosine, leucine and arginine were identified from chlamydospores of A14B but were not identified in A14 and A22 extracts. Lysine was present in races A14B and A22 but absent in A14. Results also showed that races A14 and A14B contain phosphoethanol amine and threonine which were not present in race A22 extracts. Among the 2 covered smut collections, M5 and M11, the latter contained a larger number of free amino acids than the former. Threonine, lysine, tyrosine and leucine were present in M11, but these were missing in M5. Phosphoethanol amine, histidine, and methionine were absent from chlamydospore fractions of both covered smut collections. With 1 exception these compounds were found in all loose smut chlamydospore extracts.

Lack of histidine and methionine in both covered smut collections and their presence in the 3 loose smut races was the most striking interspecific difference observed.

Among the loose smut races the 2 wide range pathogens (A14 and A14B)

Table 10. Free amino acid content of the chlamydospores of 3 loose smut races and 2 covered smut collections

A14	A14B	A22	M5	M11
Alanine	Alanine	Alanine	Alanine	Alanine
-	Arginine	-	-	-
Aspartic acid	Aspartic acid	Aspartic acid	Aspartic acid	Aspartic acid
Cysteine	Cysteine	Cysteine	Cysteine	Cysteine
Glutamic acid	Glutamic acid	Glutamic acid	Glutamic acid	Glutamic acid
Glutamine*	Glutamine*	Glutamine*	Glutamine*	Glutamine*
Glycine	Glycine	Glycine	Glycine	Glycine
Histidine	Histidine	Histidine	-	-
-	Leucine	-	-	Leucine
-	Lysine	Lysine	-	Lysine
Methionine	Methionine	Methionine	-	-
Phosphoethanol amine*	Phosphoethanol amine*	-	-	-
Serine	Serine	Serine	Serine	Serine
Threonine	Threonine	-	-	Threonine
-	Tyrosine	-	-	Tyrosine

* Compounds which react with ninhydrin.

possessed 2 compounds (phosphoethanol amine and threonine) that were not found in the narrow range pathogen (A22). This trend was also observed in the covered smut collections. The wide range pathogen (M11) contained 4 amino acids that were not found in the narrow range pathogen (M5). Therefore, there appears to be some relationship between pathogenicity patterns and amino acid content. However, the nature of relationship between nitrogen metabolism and pathogenicity was beyond the scope of this dissertation.

Bound amino acids. - Neither inter nor intraspecific differences were found in bound amino acid analysis. Results indicated that cysteine, aspartic acid, glutamic acid, serine, glycine, alanine, tyrosine, histidine, lysine, arginine, methionine, valine and phenylalanine were present in all 5 smut cultures.

Reducing sugars. - Results (Table 11) of the reducing sugar analysis showed that among loose smut races, A14 and A14B, lactose, galactose, maltose, glucose and fructose were present. Race A22 contained all of these compounds except glucose. Among covered smut collections, both M5 and M11 contained lactose, galactose, maltose and glucose. Fructose did not occur in the covered smut extracts.

Again, an interesting chemical difference was found between the loose and covered smut races used in this study---that is, all loose smut races contained fructose. This sugar was not found in the 2 covered smut collections. Moreover, the 2 wide range loose smut pathogens contained more reducing sugars than the narrow range (A22) pathogen.

Effect of host variety on free and bound amino acid contents. - Results (Table 12) of free amino acid analysis from chlamydospores obtained from a highly susceptible host (C.I. 7230) and a highly resistant host (Victorgrain) revealed that the latter contained 3 amino acids (cysteine, tyrosine, and

Table 11. Reducing sugar content of chlamydospores of 3 loose smut races and 2 covered smut collections

A14	A14B	A22	M5	M11
Fructose	Fructose	Fructose	-	-
Galactose	Galactose	Galactose	Galactose	Galactose
Glucose	Glucose	-	Glucose	Glucose
Lactose	Lactose	Lactose	Lactose	Lactose
Maltose	Maltose	Maltose	Maltose	Maltose

Table 12. Free amino acid content of chlamydospores of a loose smut collection obtained from highly susceptible (C.I. 7230) and highly resistant (Victorgrain) oat varieties

Victorgrain	C.I. 7230
Alanine	Alanine
Asparagine	Asparagine
Aspartic acid	Aspartic acid
Cysteine	-
Cystine	Cystine
Glutamic acid	Glutamic acid
Glutamine*	Glutamine*
Glycine	Glycine
Histidine	Histidine
Methionine	Methionine
Phenylalanine	-
Serine	Serine
Threonine	Threonine
Tyrosine	-
Valine	Valine

* Compounds which react with ninhydrin.

phenylalanine) not present in the C.I. 7230 extracts. Apparently, the nutrition supplied to the pathogen by the host has a considerable effect on the nitrogen metabolism of a single pathogenic race. A highly susceptible host propagates a wider range of pathogenic biotypes than a host which is susceptible to a few specialized races. Therefore chlamydospores from C.I. 7230 should contain a wider range of amino acids than those from Victorgrain. Results were contrary to this hypothesis; therefore the effect of host variety on the evolution of pathogenicity warrants little speculation when expressed in terms of amino acid differences only. This conclusion does not alter the fact that host variety affects nitrogen metabolism of the pathogen, which in turn may play a role in the parasitism of the parasite.

Both kinds of chlamydospores, i.e. those obtained from Victorgrain and C.I. 7230 oat varieties, contained the same bound amino acids. These bound amino acids were---cystine, aspartic acid, glutamic acid, serine, glycine, alanine, tyrosine, histidine, methionine, lysine, valine, arginine and phenylalanine. These protein amino acids were also present in races $A1^{4}$, $A1^{4B}$ and A22 of U. avenae and collections M5, M11 of U. kolleri. Apparently, the bound amino acid content of oat smut species is not affected by the host on which they are propagated. Neither interracial nor interspecific differences are expressed in the bound amino acid content of oat smut species. Therefore, the protein metabolism of these fungi is somewhat similar.

Two distinct trends were observed throughout the chemotaxonomic study, i.e. wide range pathogens contain more free amino acids and reducing sugars than do narrow range pathogens, regardless of the species studied. Secondly, there was a consistent interspecific

difference in the free amino acids and reducing sugars content of the races studied. It is tempting to speculate on the role that these differences play in pathogenicity and species evolution. However, studies should be extended to include other races of these 2 species before firm pathologic or taxonomic relationships can be established.

SUMMARY

Pathologic, physiologic, genetic and chemotaxonomic relationships between races of oat smut species (Ustilago avenae and U. kolleri) were studied. Loose smut races A14 and A14B are wide host range pathogens and A22 is a narrow range pathogen. Covered smut collection M11 is a wide range and M5 is a narrow range pathogen.

When chlamydospores of A14, A22, M5 and M11 were germinated on 5 agar media it was found that loose smuts germinated slower than the covered smuts. The effect of culture media on spore germination demonstrated that loose smut races and one of the covered smut collections germinated at different rates on different media. Collection M5 germinated well on all media. The optimum temperature range for chlamydospore germination was 24° to 32° C. No interaction between temperature and culture media was observed. Growth studies indicated that potato dextrose-malt extract agar was most suitable for the growth of all cultures except collection M11 which grew best on Blakeslee's agar. The pronounced increase of growth of 3 cultures on potato dextrose-malt extract agar was attributed to the balanced nutrition of this medium. All 4 smut cultures grew most vigorously on Blakeslee's liquid (shake) medium. Cultural studies revealed no interaction between nutritional requirements for growth and host range.

The effect of spore age and culture media on pathogenicity of

races A14 and A22 was determined. Progeny (F_2) from interracial hybrids and selfed spores which were grown on potato dextrose-Victorgrain extract agar for 20 days gave the highest degree of smut infection. Results indicated that increased pathogenicity was due to the balanced nutritional factors of potato dextrose-Victorgrain extract agar. Twenty day old spores were more infectious than 10 or 30 day old spores.

The effect of infection by both smut species on growth of the host was determined at periodic intervals for 40 days. Race A14 decreased growth and M5 increased growth of the same variety. Race A22 reduced the growth rate of Red Rustproof-14 but it increased the growth of Victorgrain. Victorgrain expressed a higher smut infection than the Red Rustproof-14. Therefore, the increased growth which was significant at the 1% level, may be due to the excretion of an endogenous growth regulator by the pathogen or an alteration of the host metabolism.

Inheritance of reaction of F_2 plant families derived from a cross between Fulgrain 3 and Fulgrain 7 to loose smut races A20 and A22 was determined. Fulgrain 3 is susceptible to A22 and Fulgrain 7 is susceptible to A20. Tests which were conducted under field conditions consisted of 100 F_2 plant families. Analysis (χ^2) indicated that resistance to A20 is dominant whereas resistance to A22 is recessive. This is the first report that resistance to loose smut is controlled by recessive gene action. Inheritance of host reaction to A20 and A22 is monogenic. Genes controlling the reaction to these 2 pathogens were inherited independently.

Chemotaxonomic studies indicated inter and intraspecific differences in free amino acids and reducing sugars content of chlamydospores of the species studied. Wide range pathogens contained more free amino acids

and reducing sugars than narrow range pathogens. Loose smut races contained 2 free amino acids which were not present in covered smut collections. Bound amino acids were the same in all 5 smut cultures. Variable numbers of free amino acids were identified from chlamydospores of a loose smut collection from a highly susceptible and a highly resistant oat variety. Therefore, host variety affected the nitrogen metabolism of the invading pathogen, but did not change the bound amino acid content.

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BIOGRAPHICAL SKETCH

Avdhesh Narain was born on February 24, 1934, at Lucknow, Utter Pradesh, India. In May, 1949, he was graduated from Government Jubilee High School, Lucknow; he received Intermediate in Science diploma from Government Jubilee College, Lucknow, in May, 1951. In the fall of 1951 he enrolled in the Science Faculty of Lucknow University and received the degree of Bachelor of Science in 1953. He further continued his studies for graduate work at Lucknow University and obtained the degree of Master of Science in Botany in the year 1955. From 1955 until July, 1958, he worked as a Research Assistant on an Utter Pradesh Government research project at Lucknow University. In September, 1958, he enrolled at the University of Florida; in the fall of 1960 he was appointed as a Research Assistant in the Department of Plant Pathology and until the present time he has pursued work toward the degree Doctor of Philosophy.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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